Genetically encoded fluorescent sensors for calcium (GCaMP) glutamate (iGluSnFR) and GABA (iGABASnFR)

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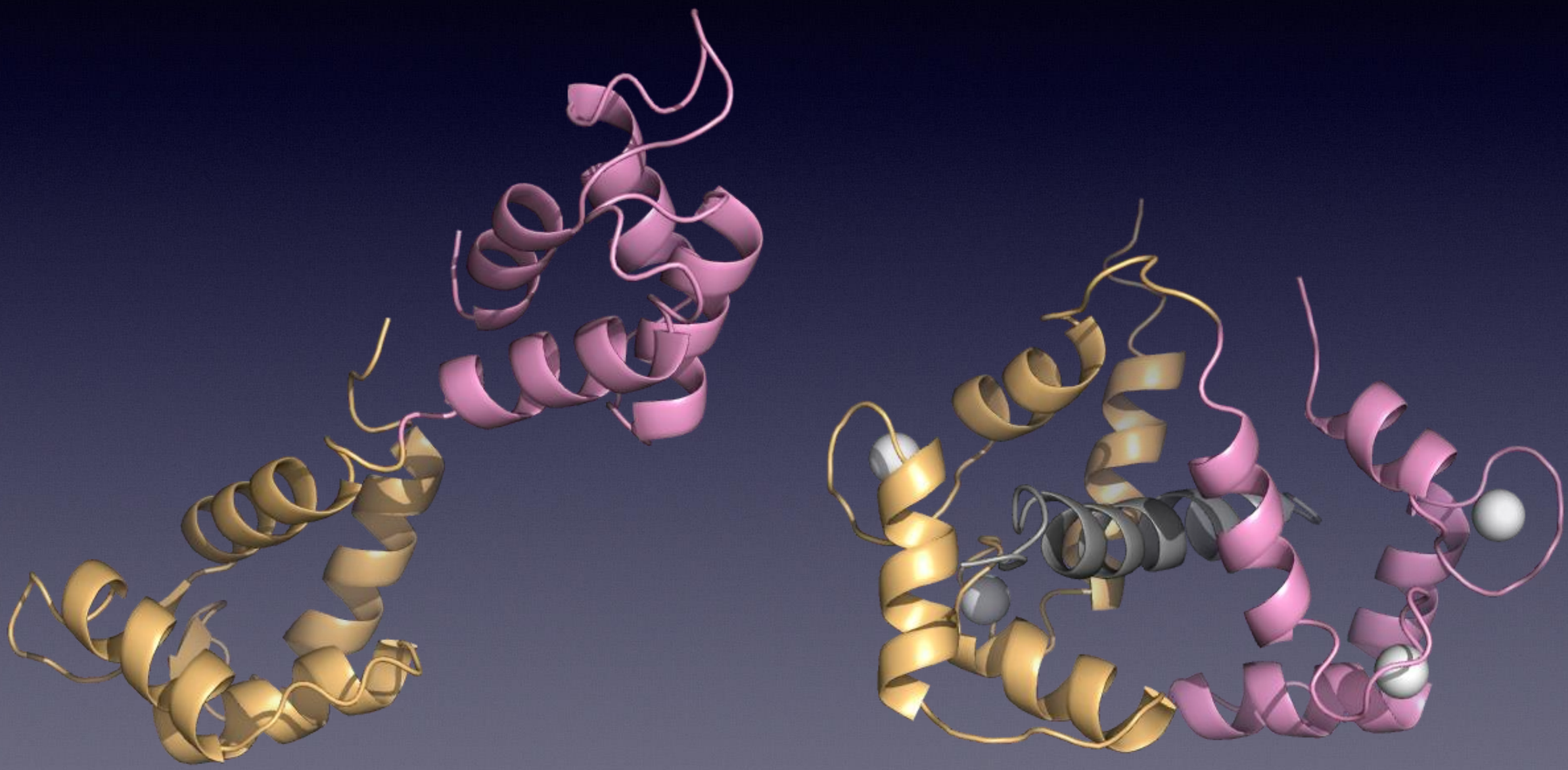
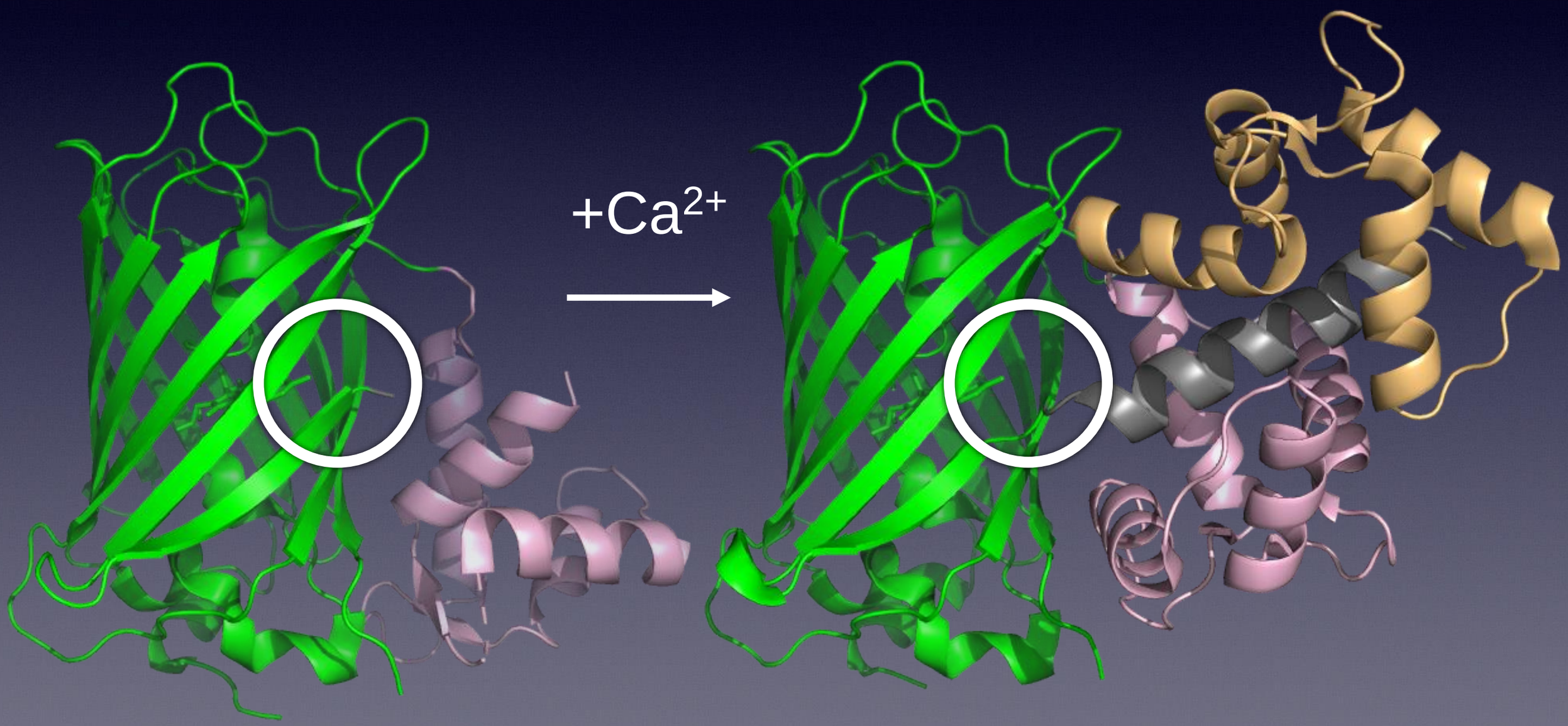
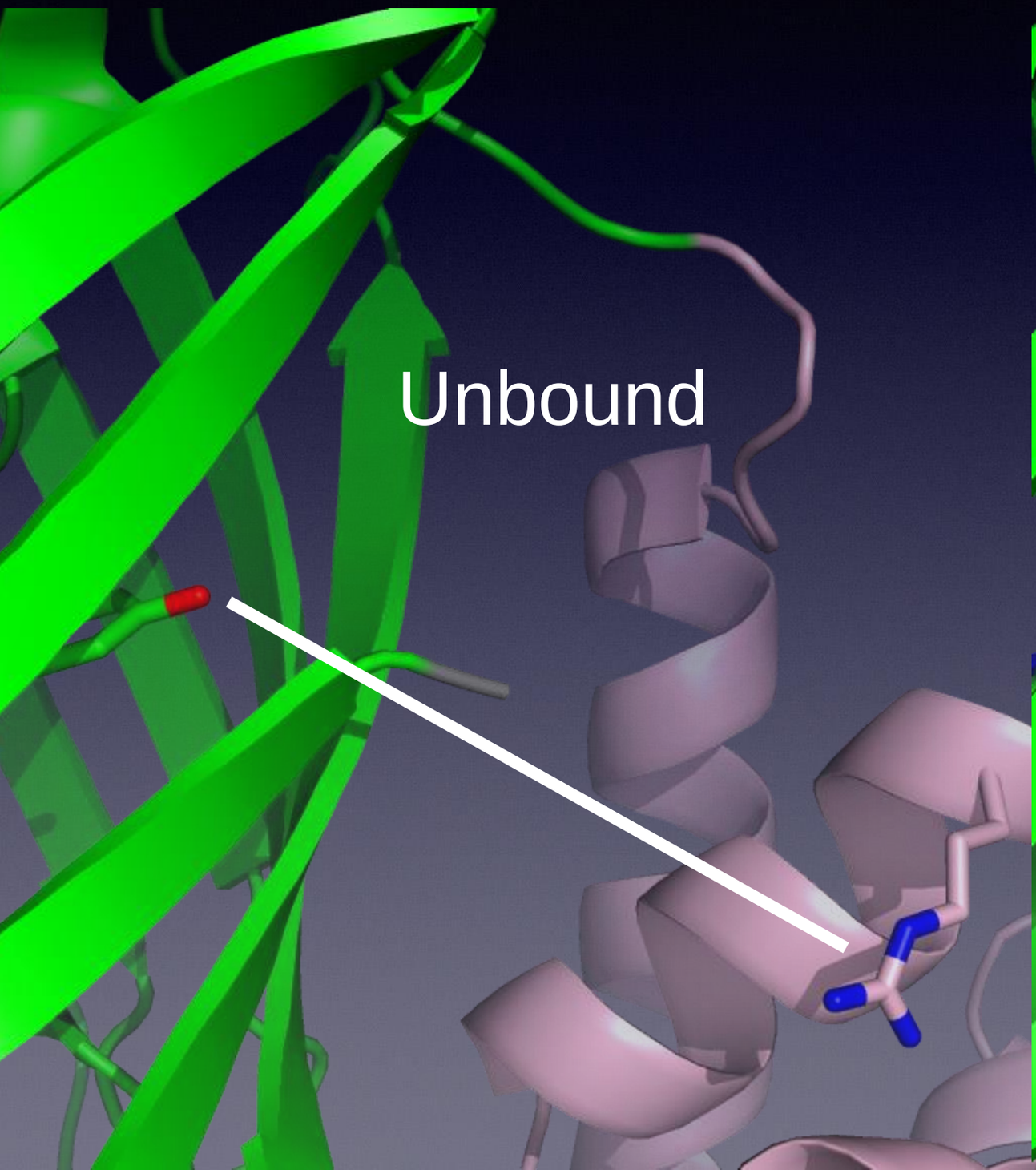
Ligand-induced conformational changes

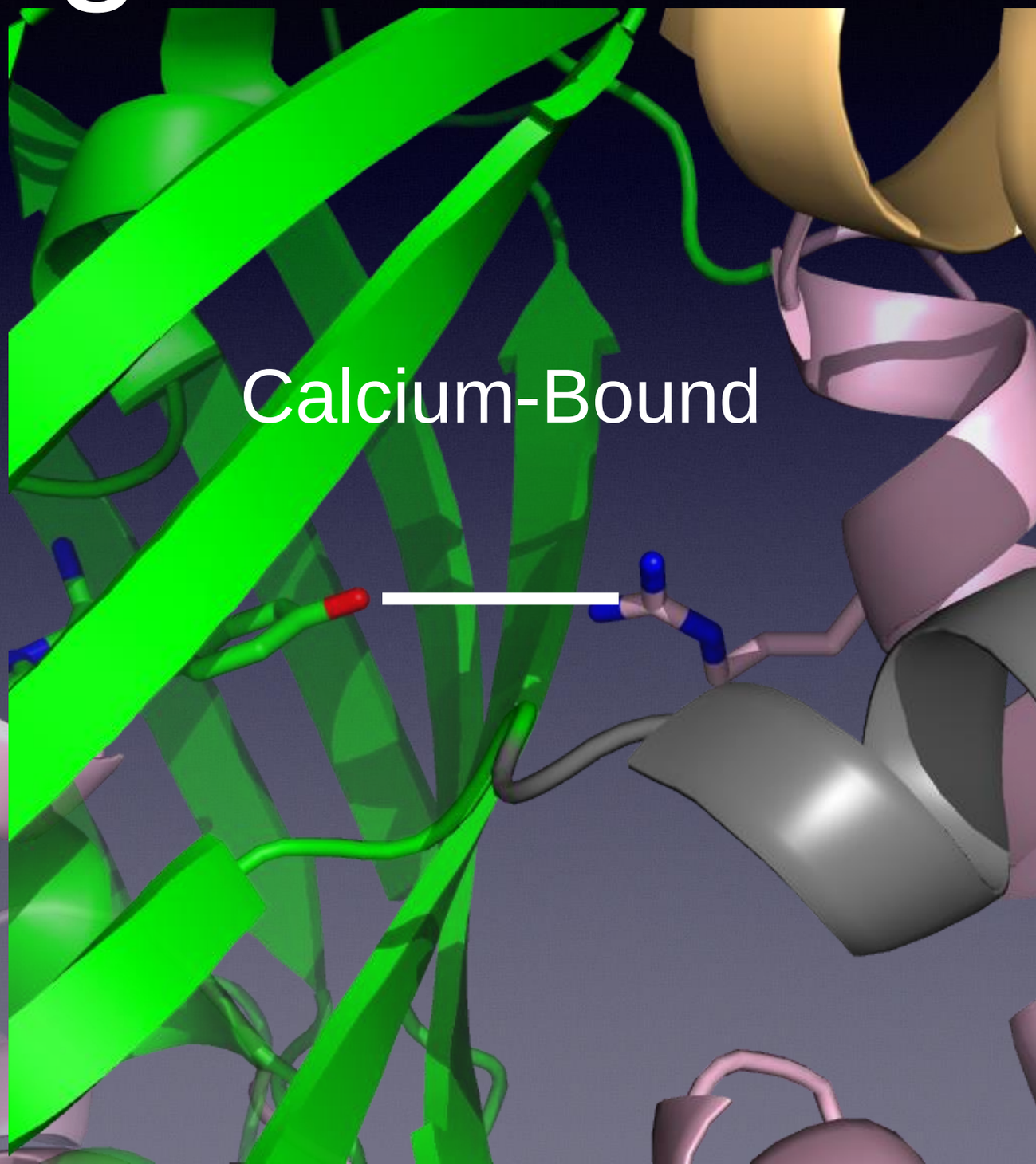


Ligand-induced conformational changes coupled to fluorescence in GCaMP



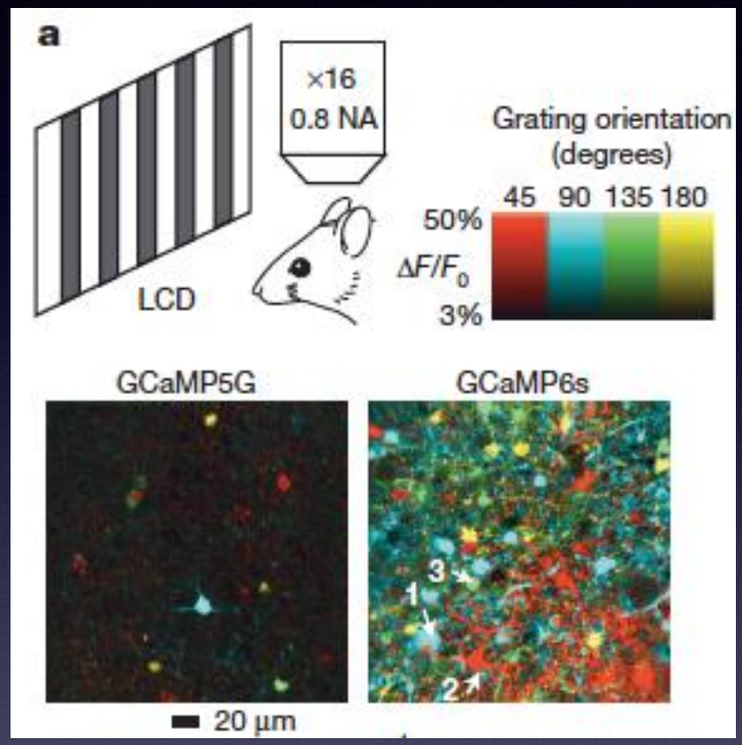
Local environmental changes

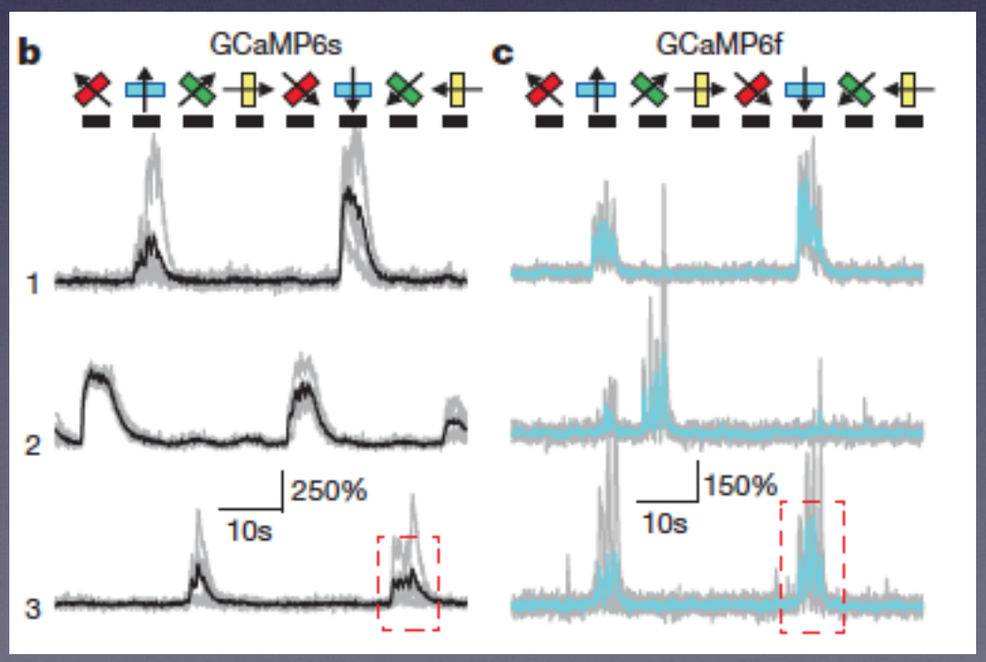


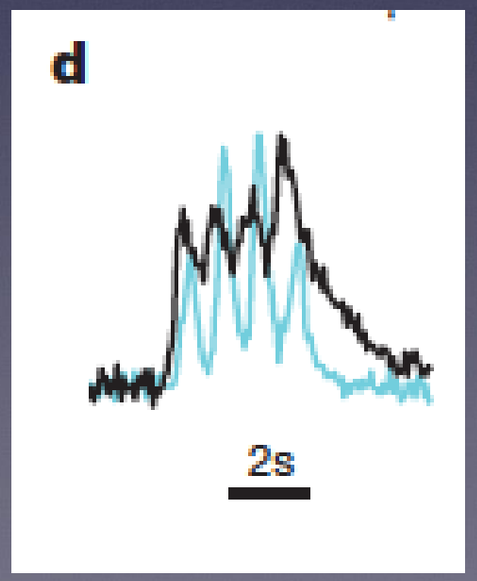


GCaMP can resolve individual calcium spikes

"Best data"
- Orientation-selective activity can be identified in 70% of visual cortex cells





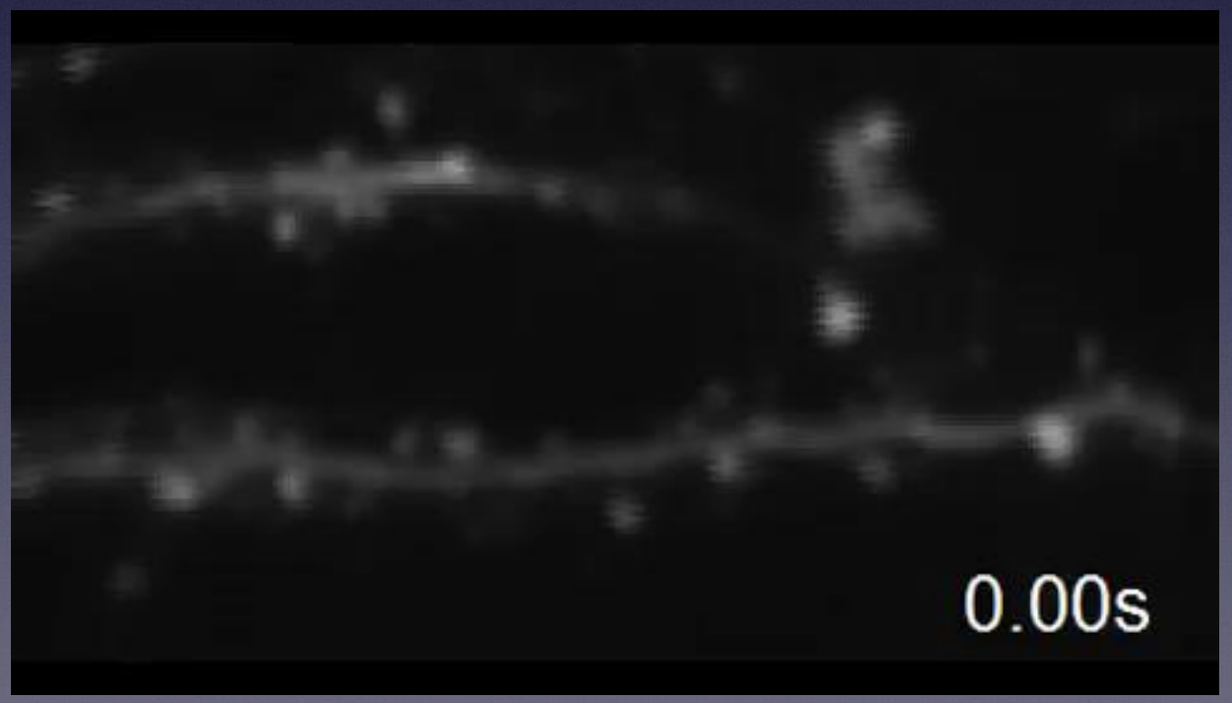


Resolution of individual spikes

1. Chen, T.-W. *et al.* Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499,** 295–300 (2013).

…even at individual spines

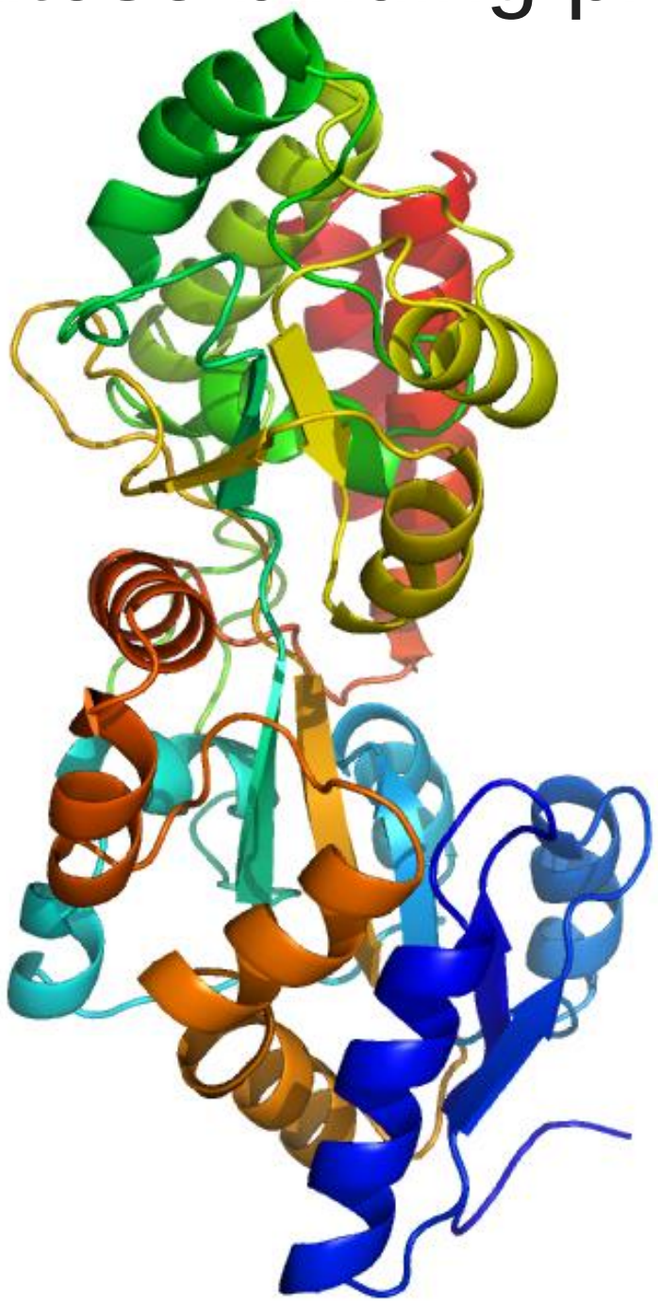
Visual stimulus test, mouse visual cortex



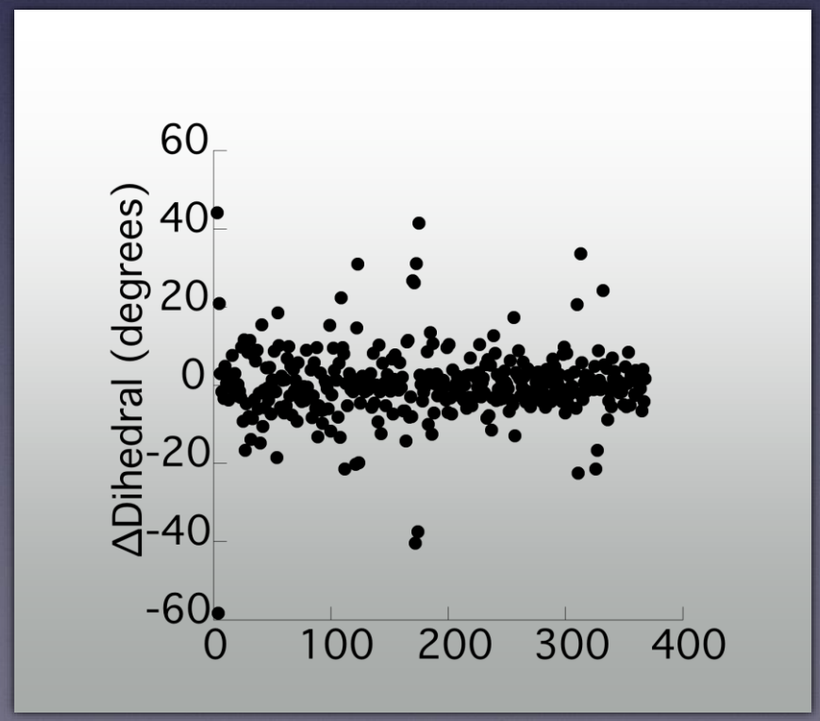
1. Chen, T.-W. *et al.* Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499,** 295–300 (2013).

Making sensors like GCaMP, but for other stuff

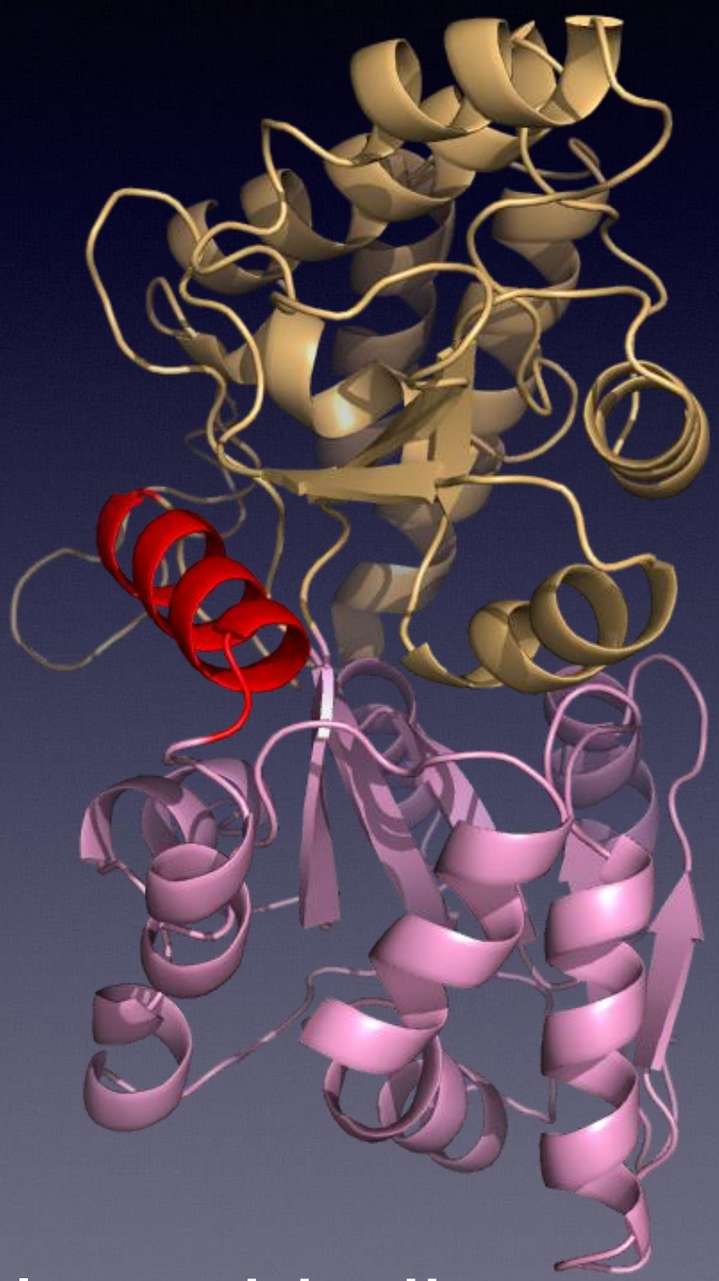
Maltose binding protein



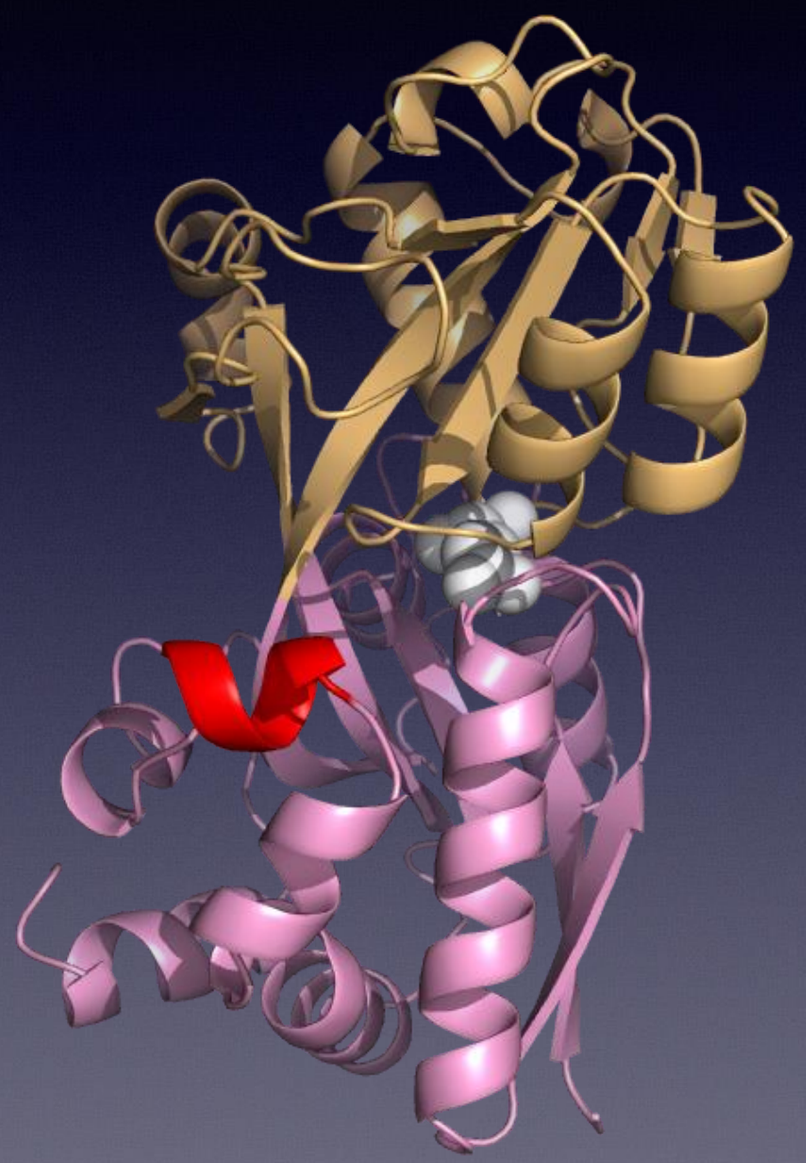
- Venus-flytrap like binding motion
- Identify local conformational changes
- Insert cpGFP and optimize



iGluSnFR design
intensity-based glutamate sensing fluorescence reporter



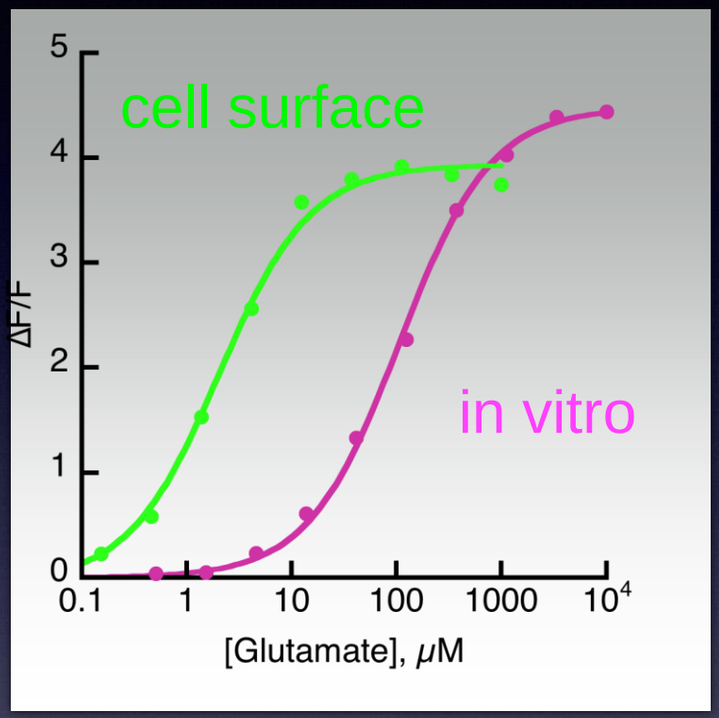
Maltose binding protein

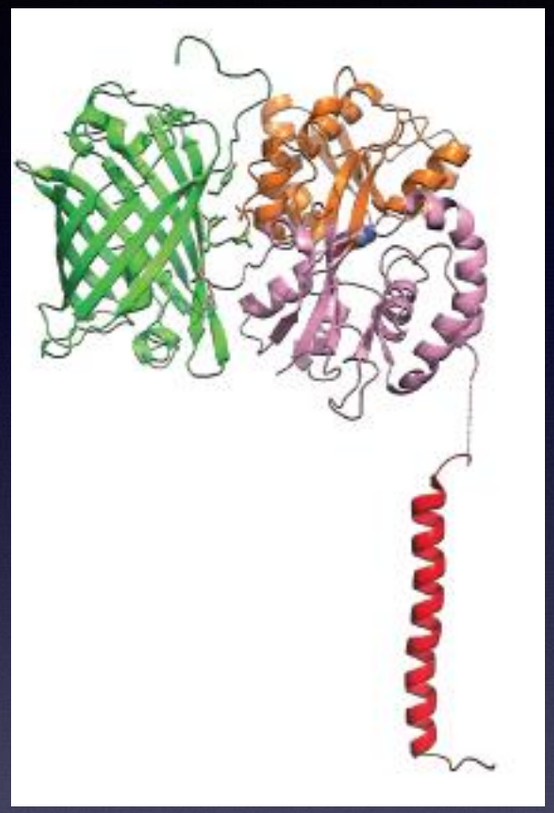


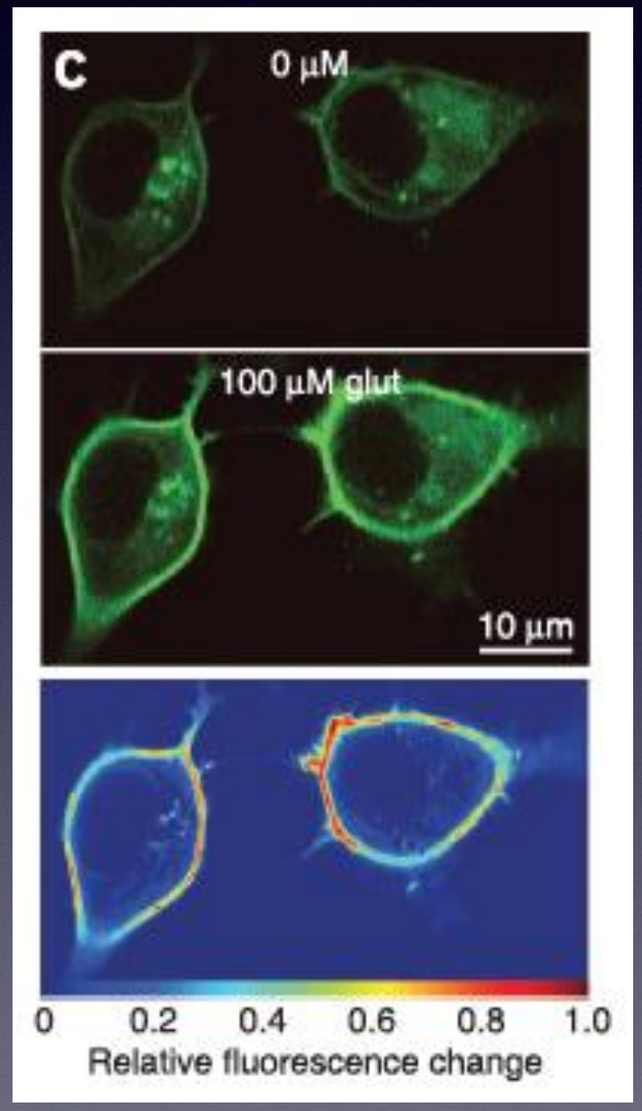
Glutamate binding protein

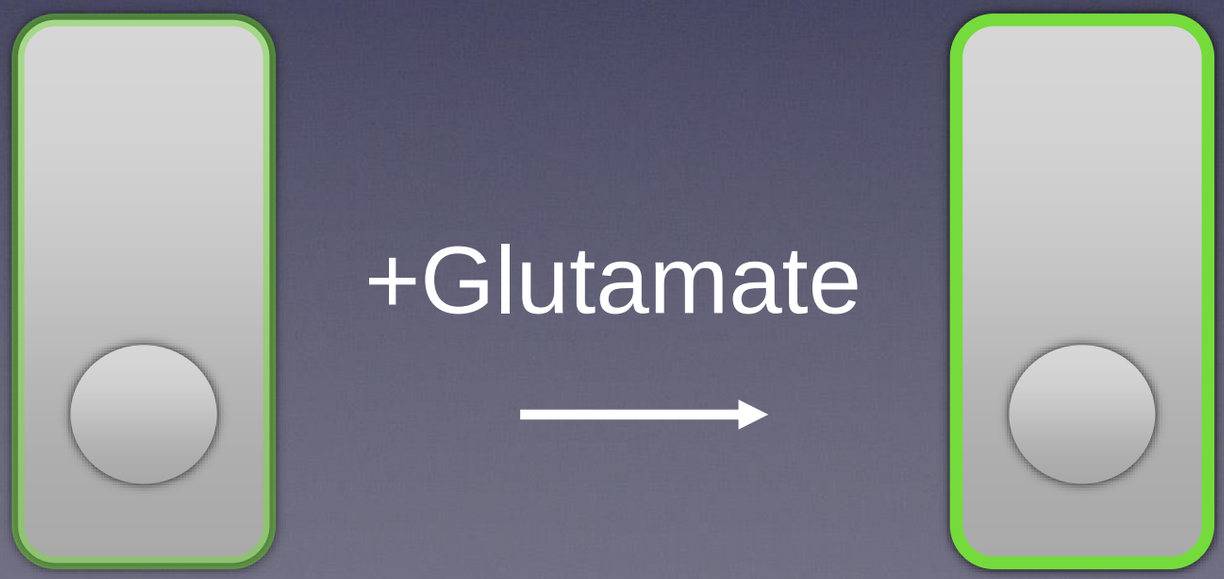
Details about optimization

iGluSnFR to detect synaptic neurotransmission

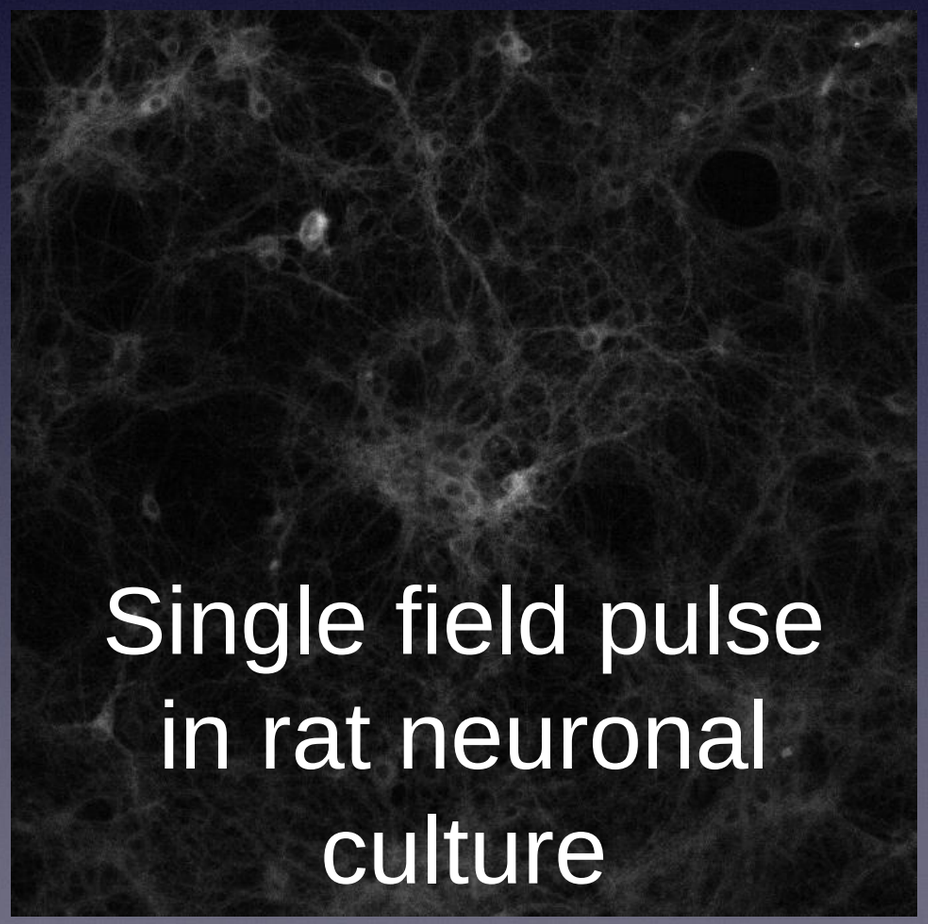




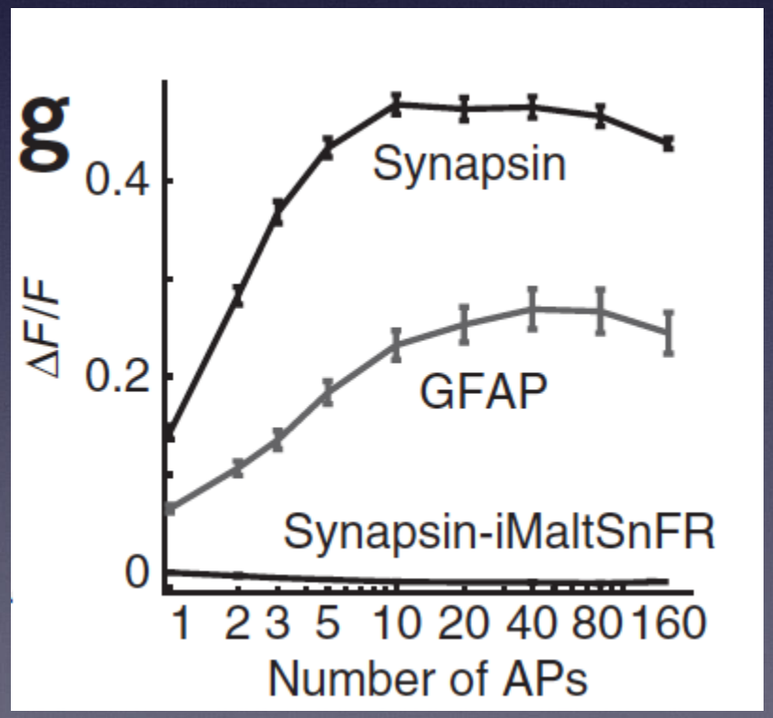




iGluSnFR in neuronal culture

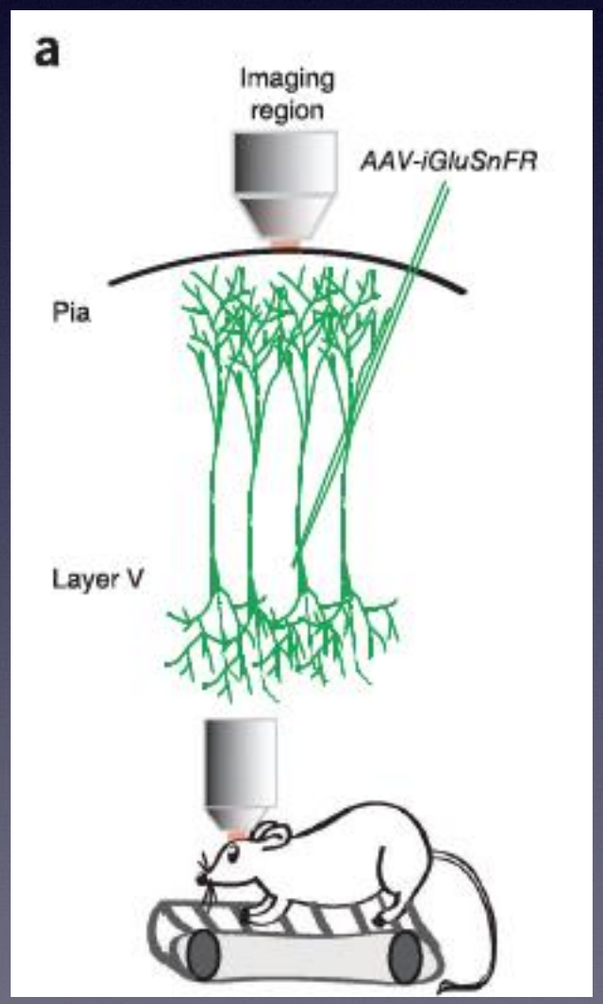


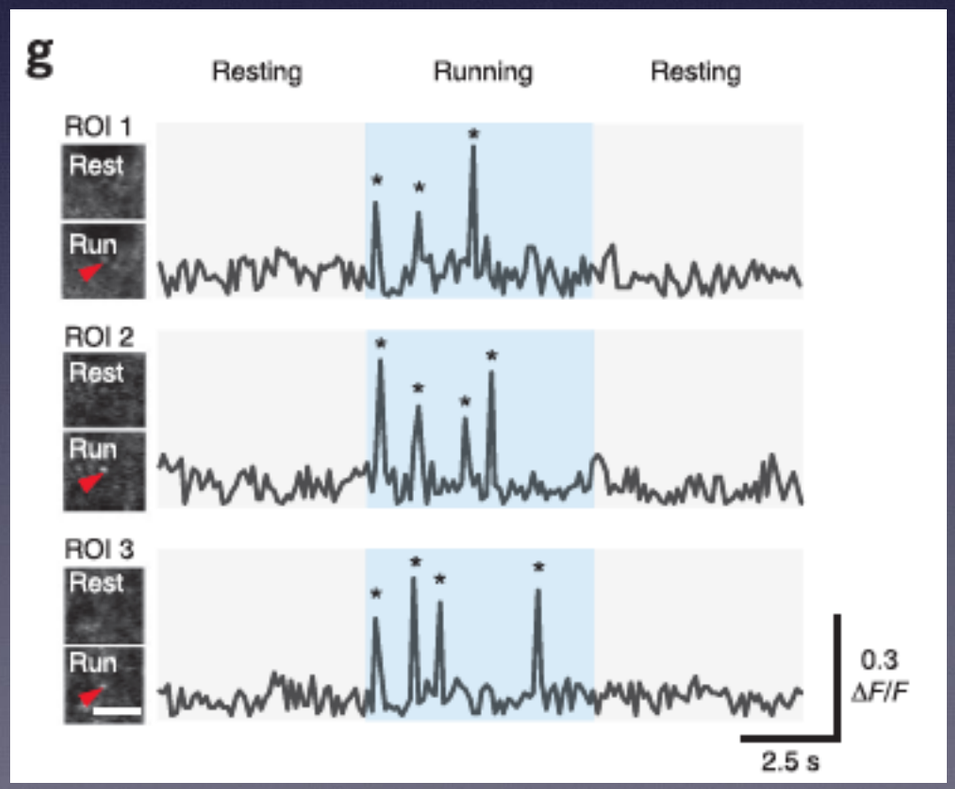
Single field pulse in rat neuronal culture



iGluSnFR performance

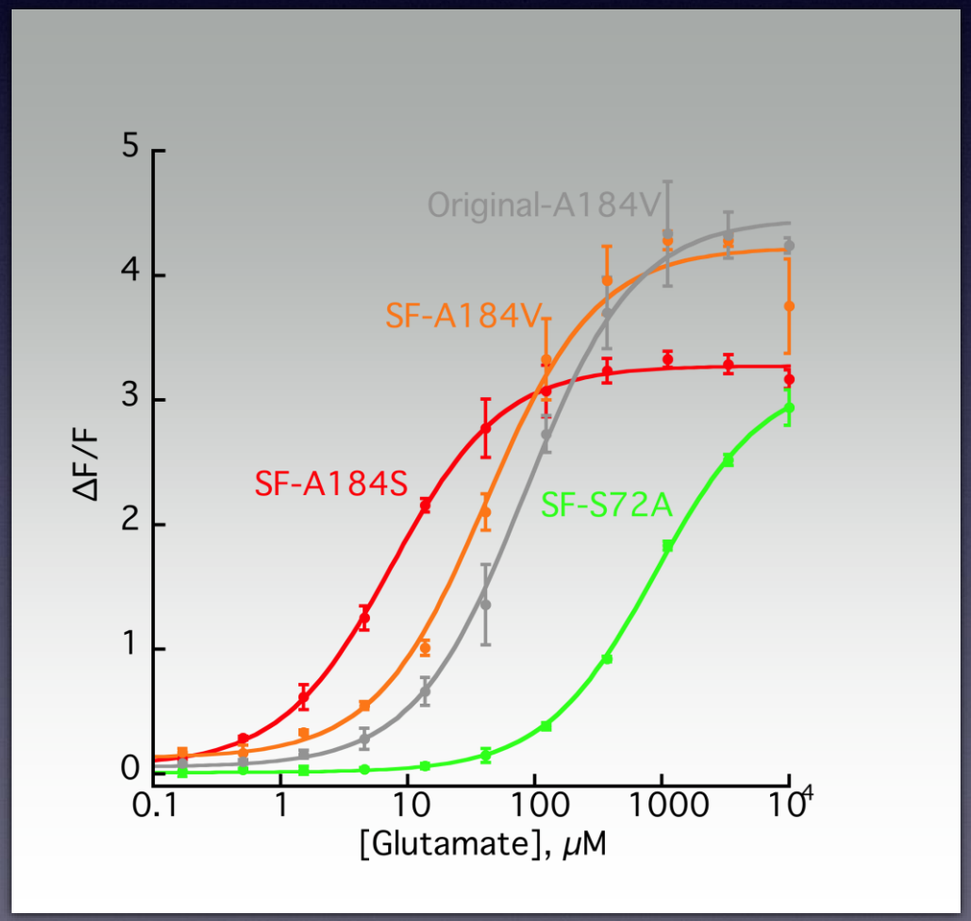
Highlight: observing activity dependent changes in mouse motor cortex

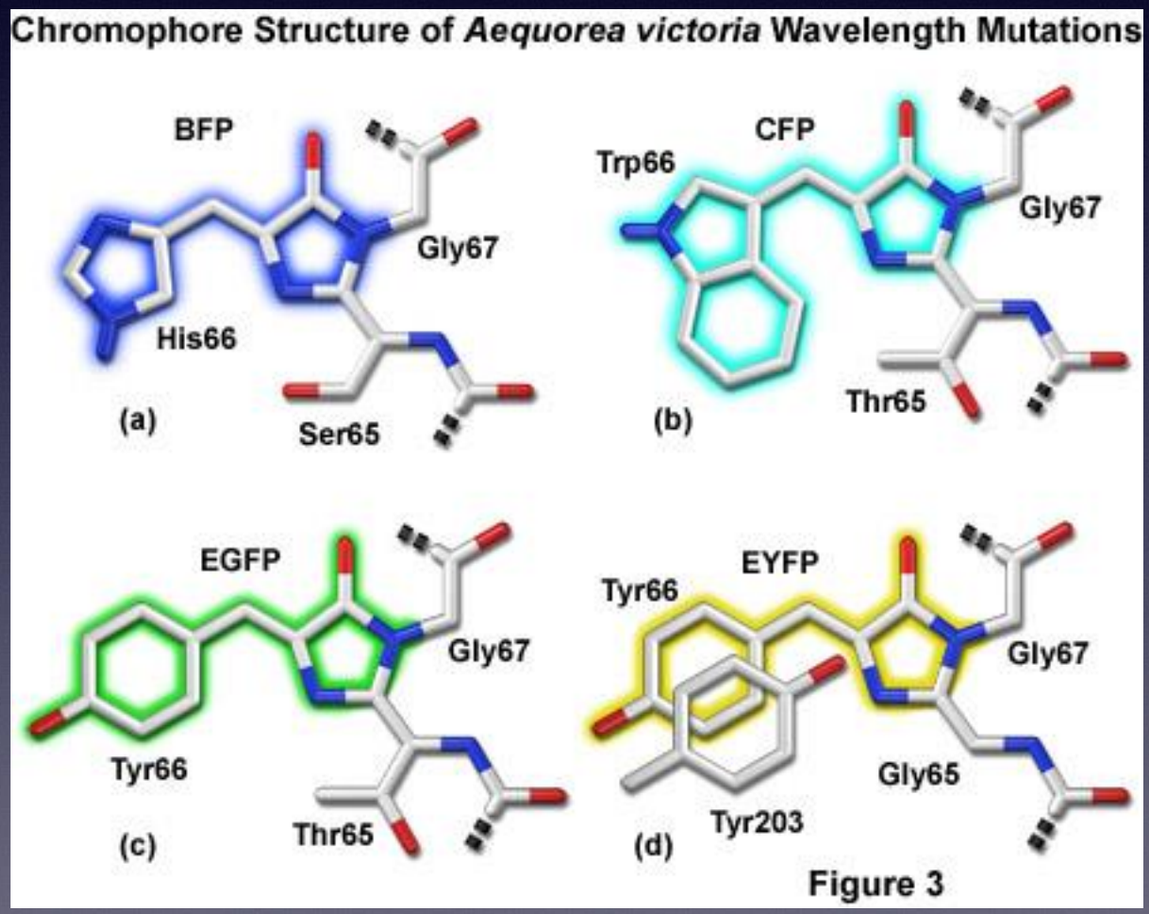




Joe Cichon @ NYU

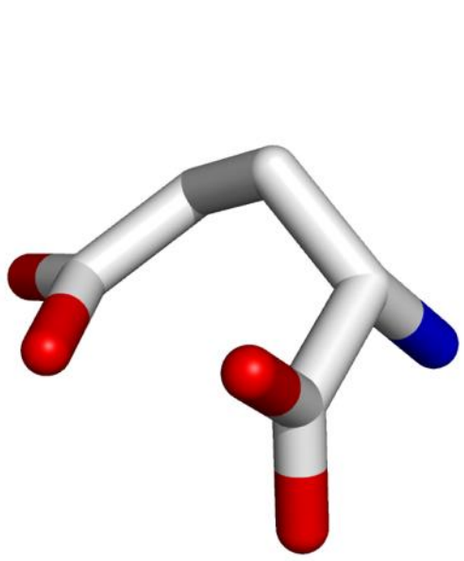
iGluSnFR comes in different affinities and colours



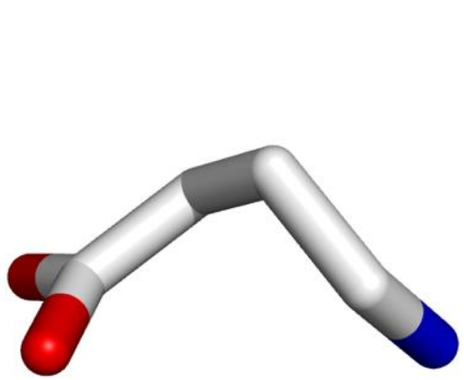


iGABASnFR

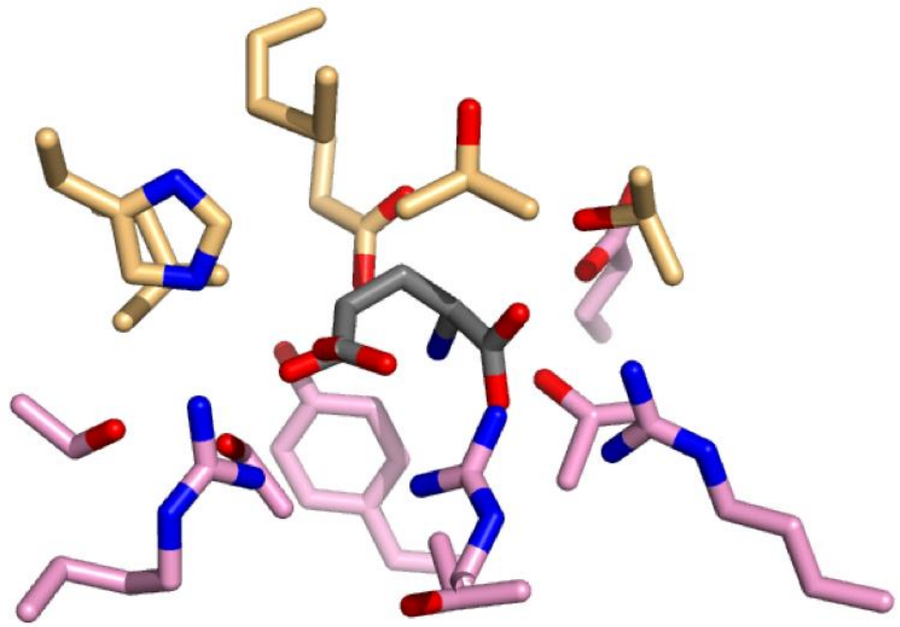
Redesign iGluSnFR?



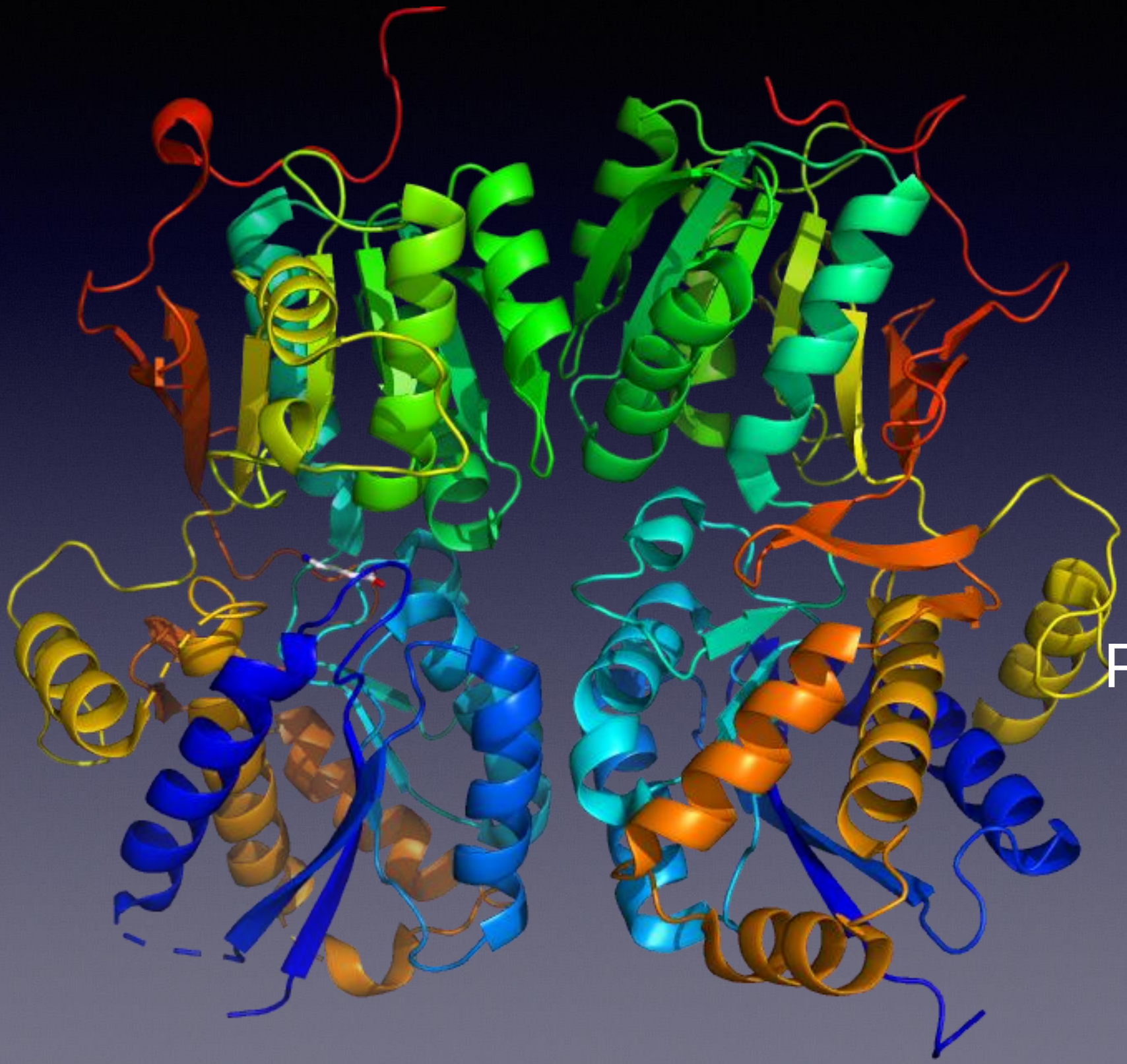
Glutamate



GABA



iGABASnFR scaffold



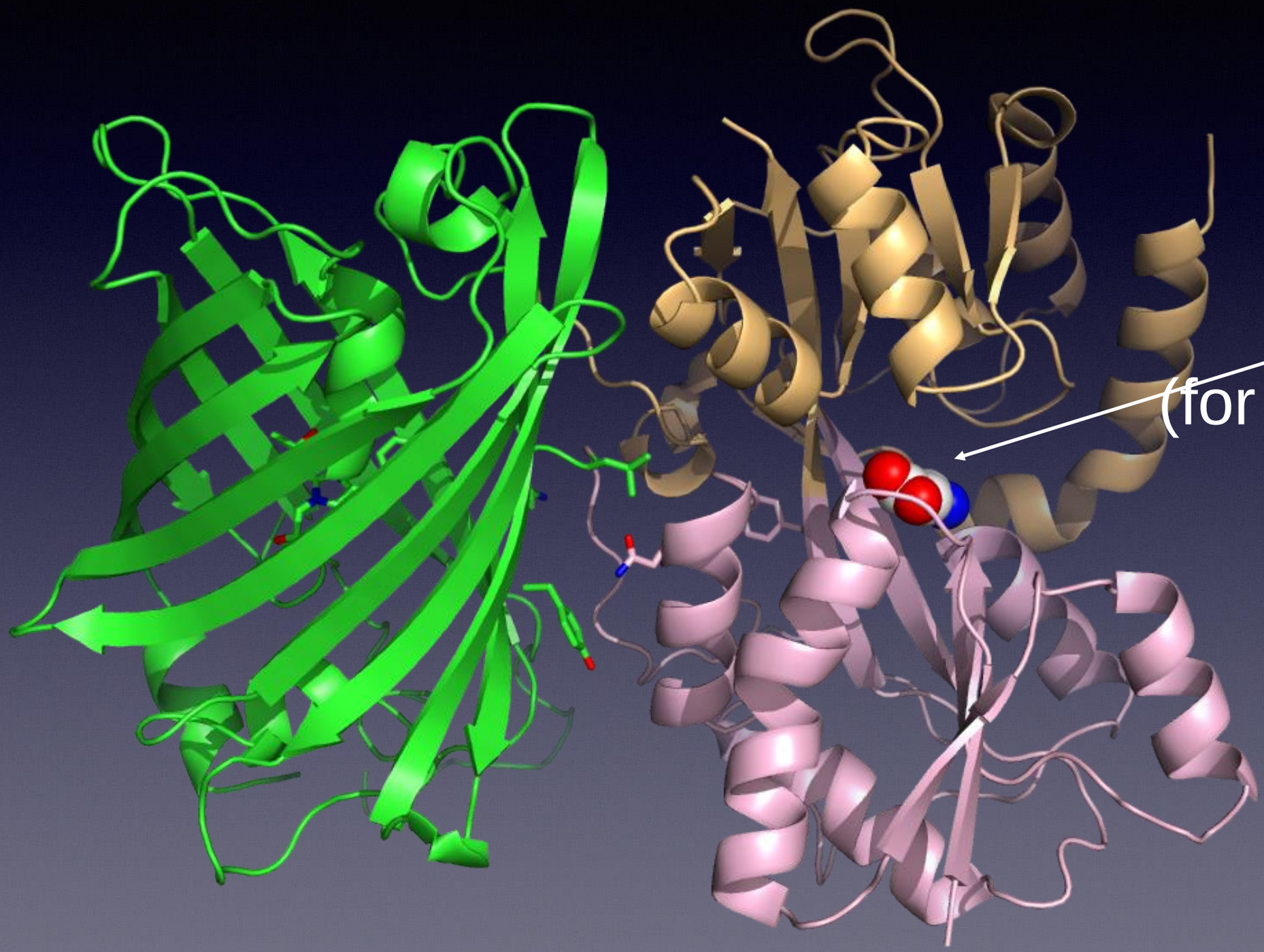
GABA(B) receptor

Heterodimer

Difficult to express

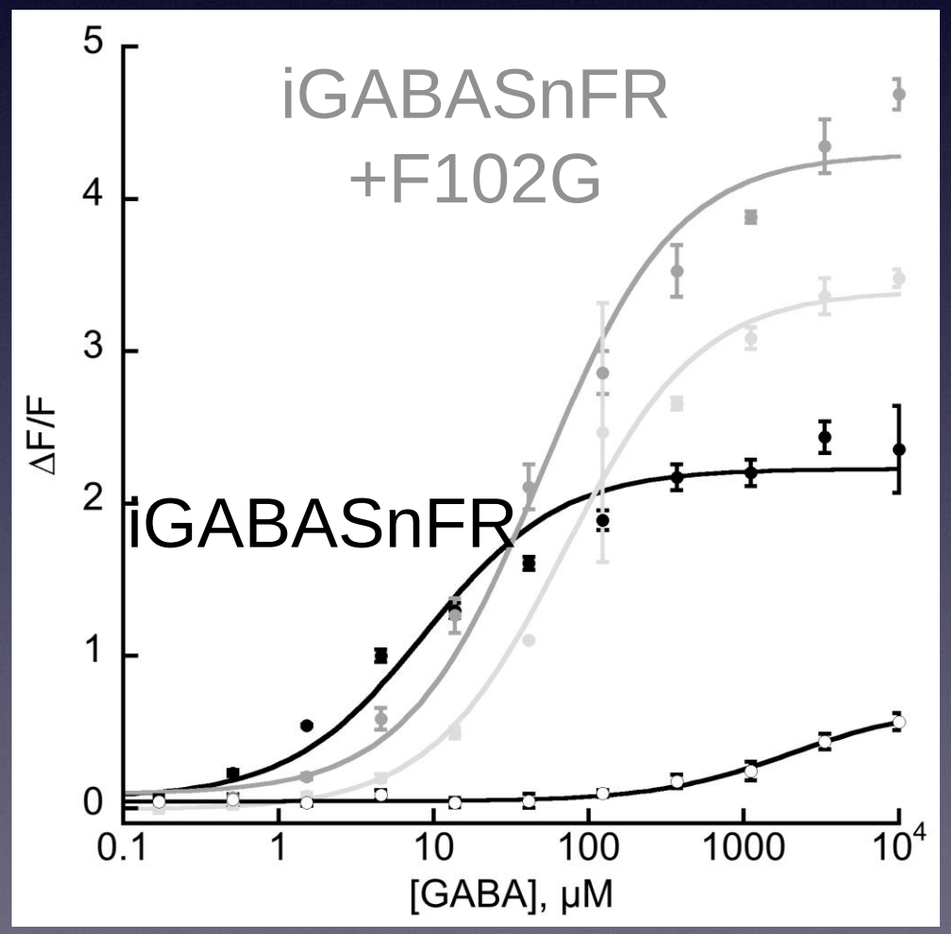
Potential for adverse interactions

iGABASnFR

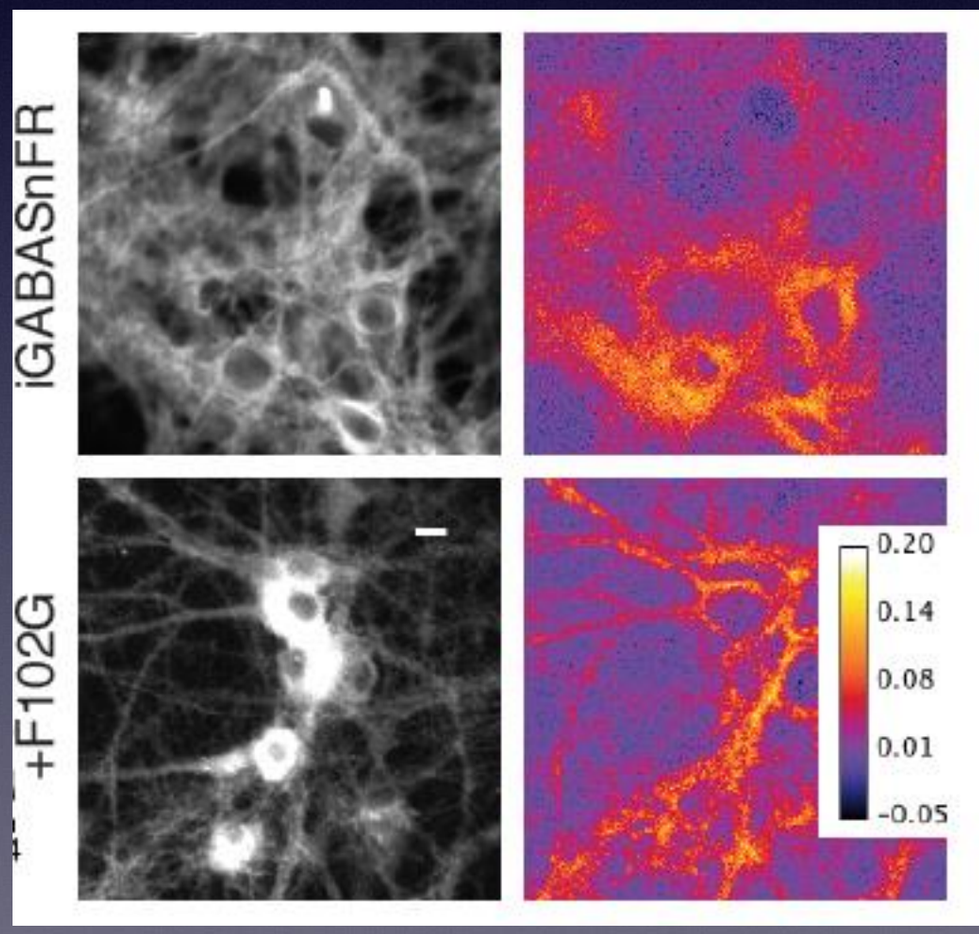


Multi-dimensional “improvement” elusive

In vitro

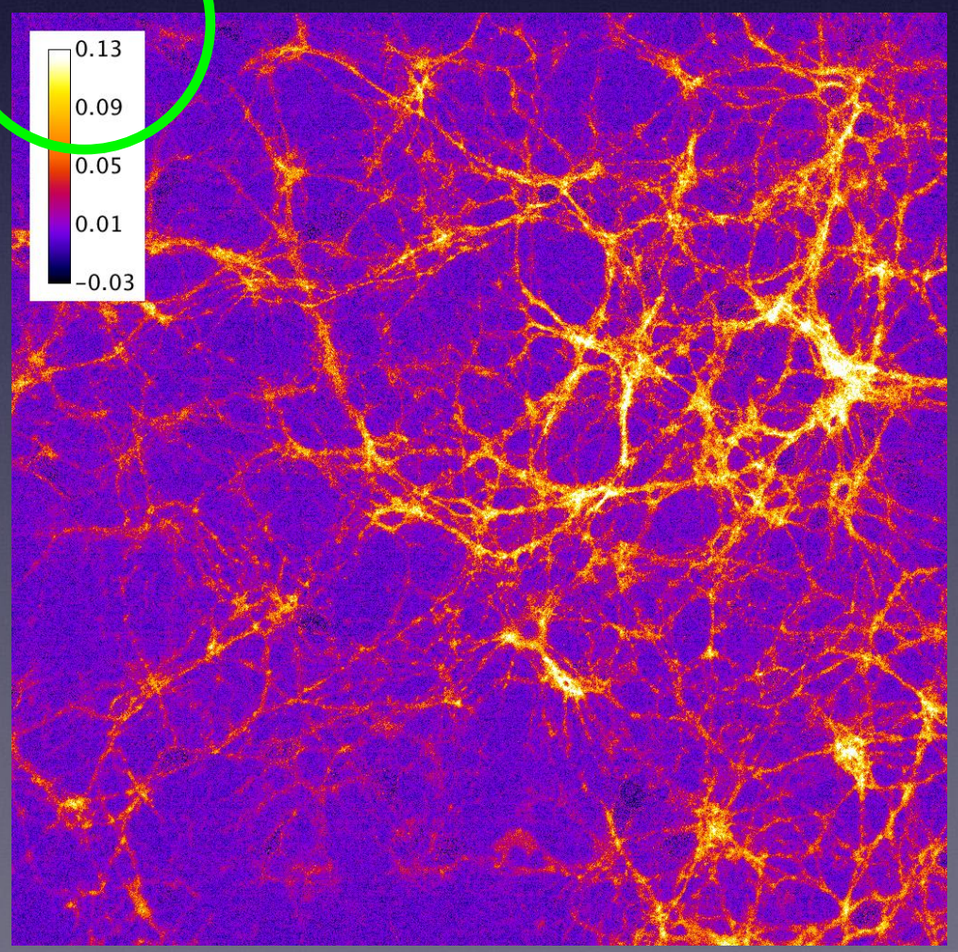


Neuronal culture,
40 electrical stimuli

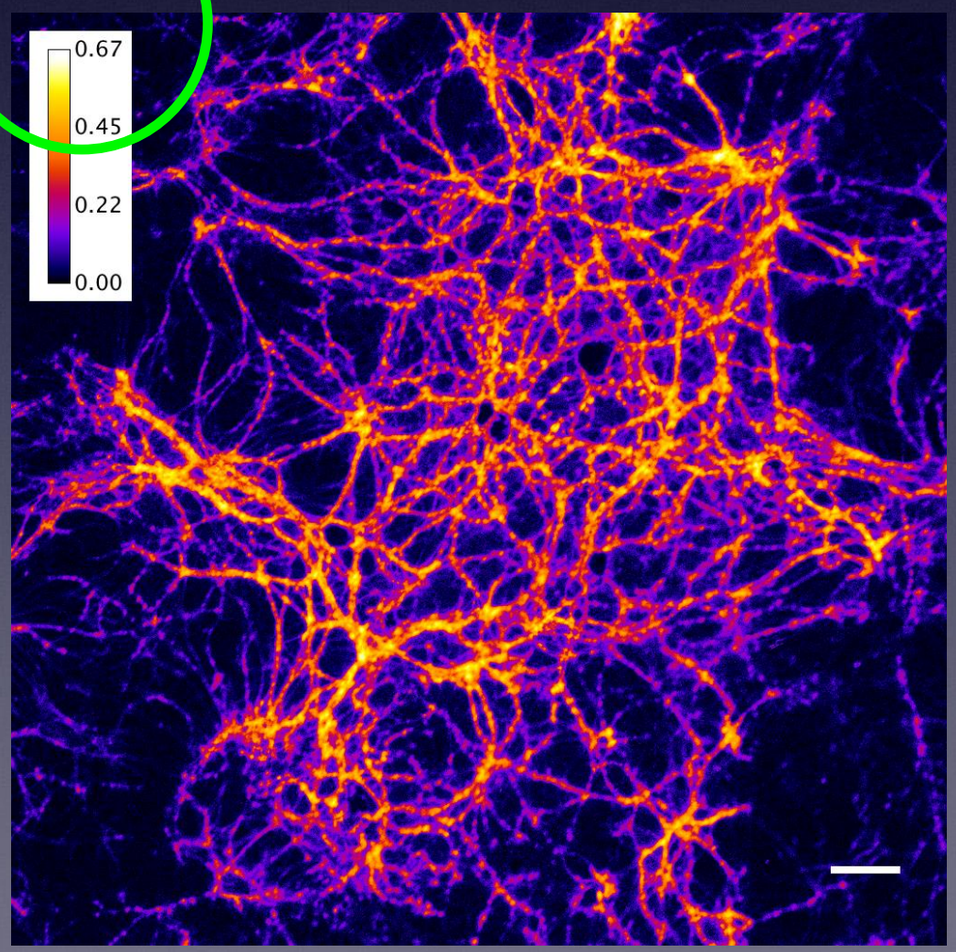


Not as robust as iGluSnFR

iGABASnFR

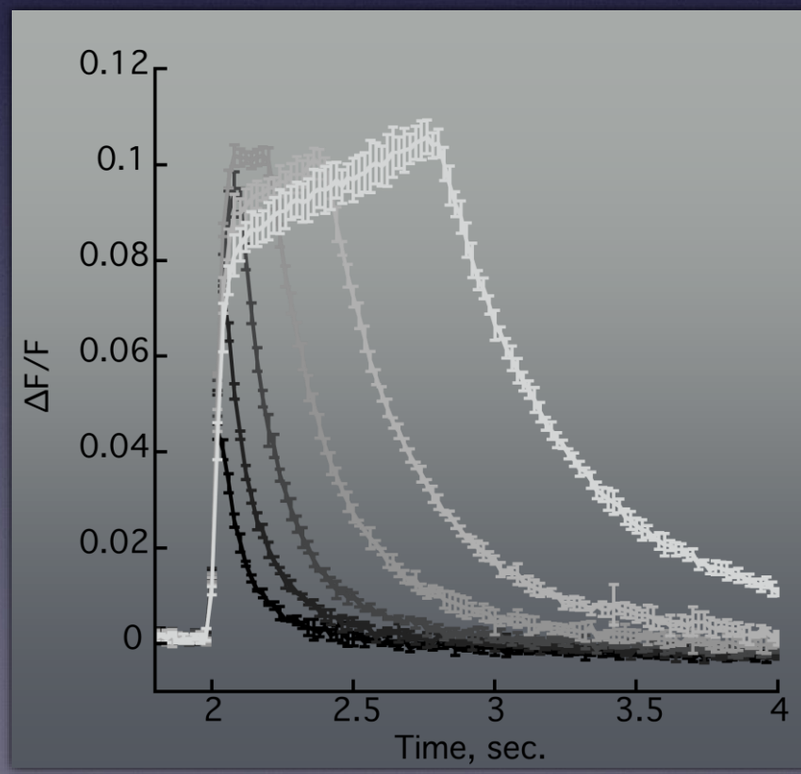


iGluSnFR

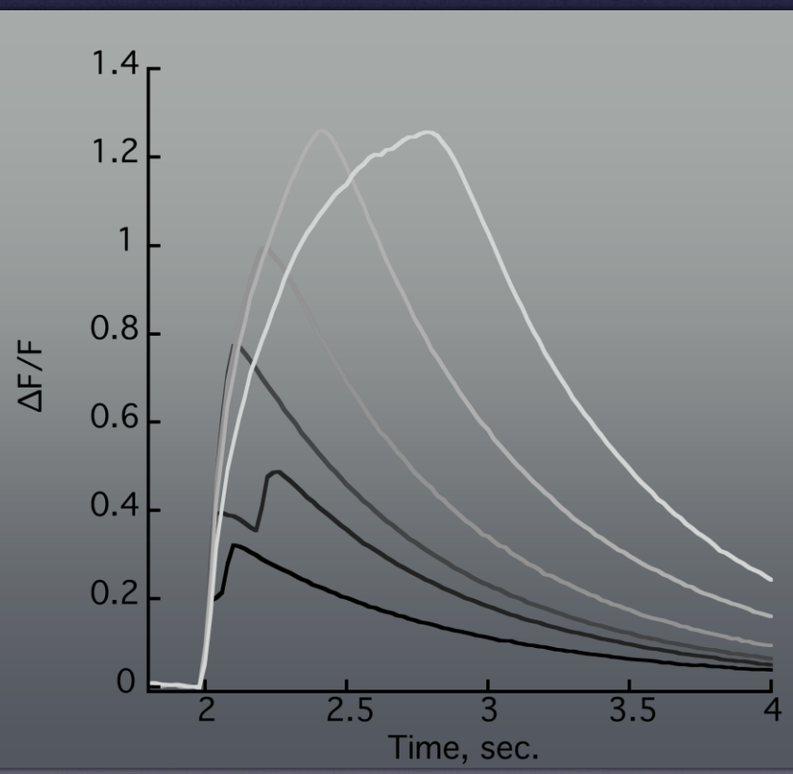


Not as robust as iGluSnFR

iGABASnFR.F102G

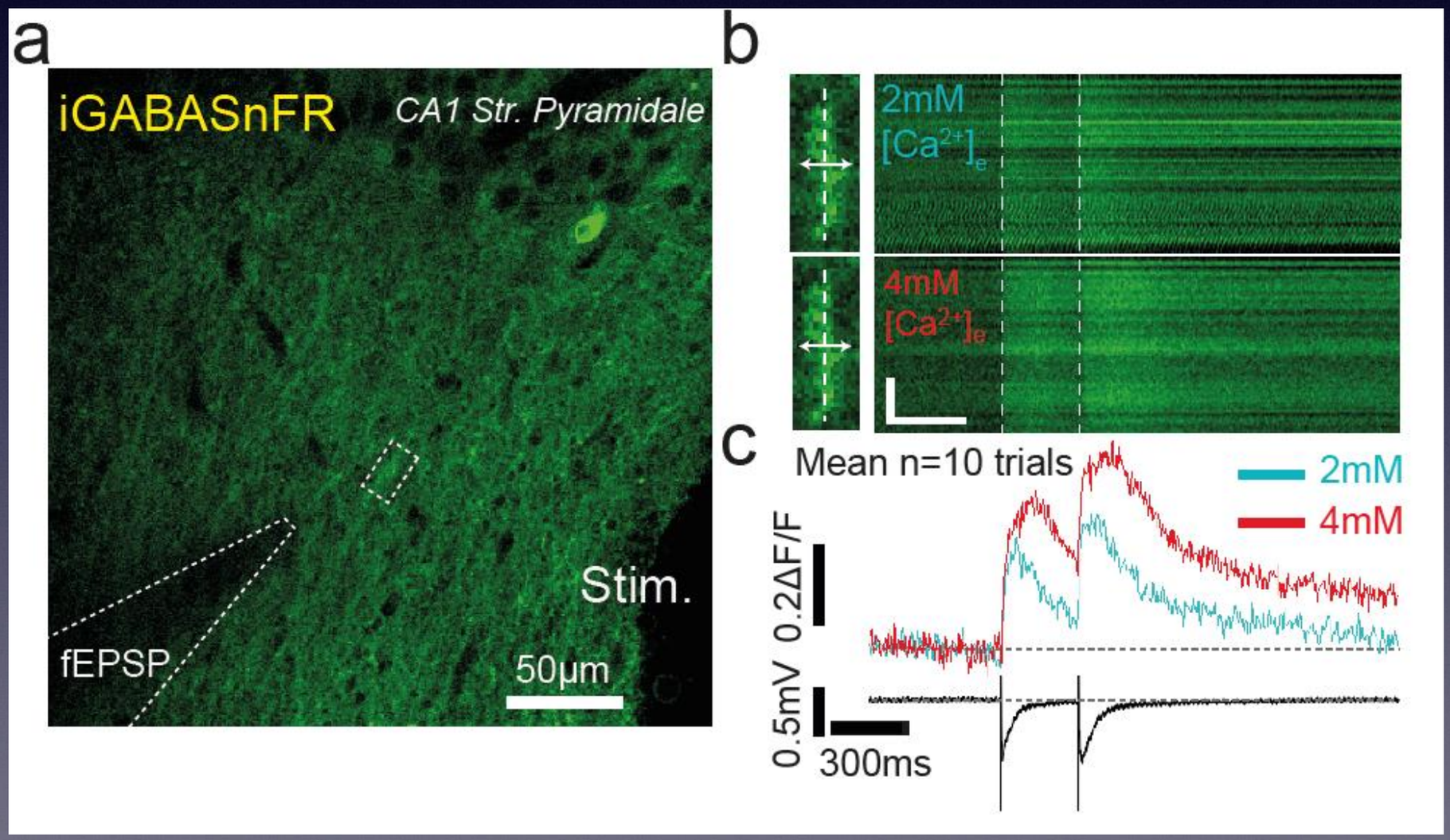


SF-iGluSnFR

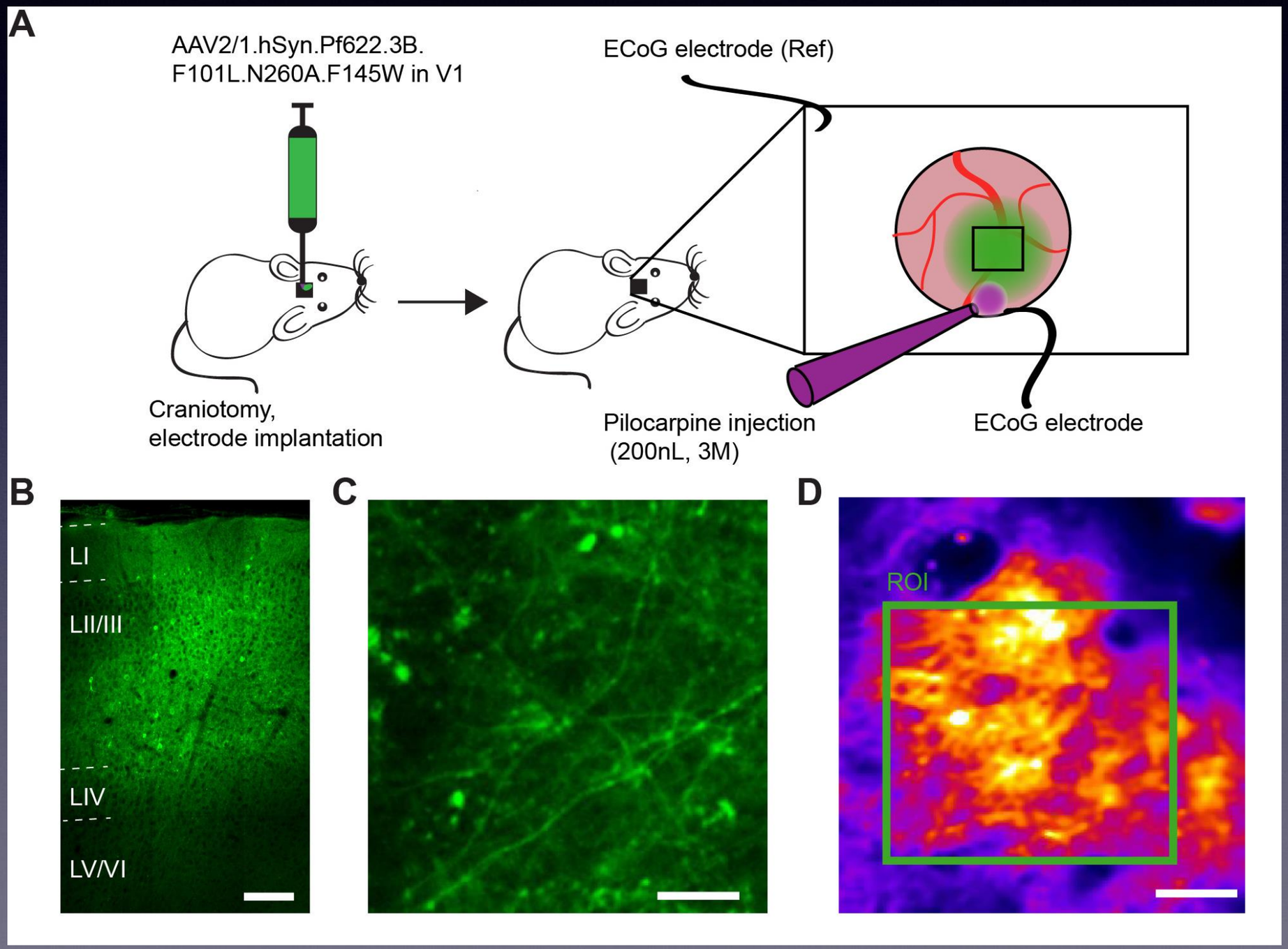


iGABASnFR responds in hippocampal slice

- Increasing [calcium] increases release probability

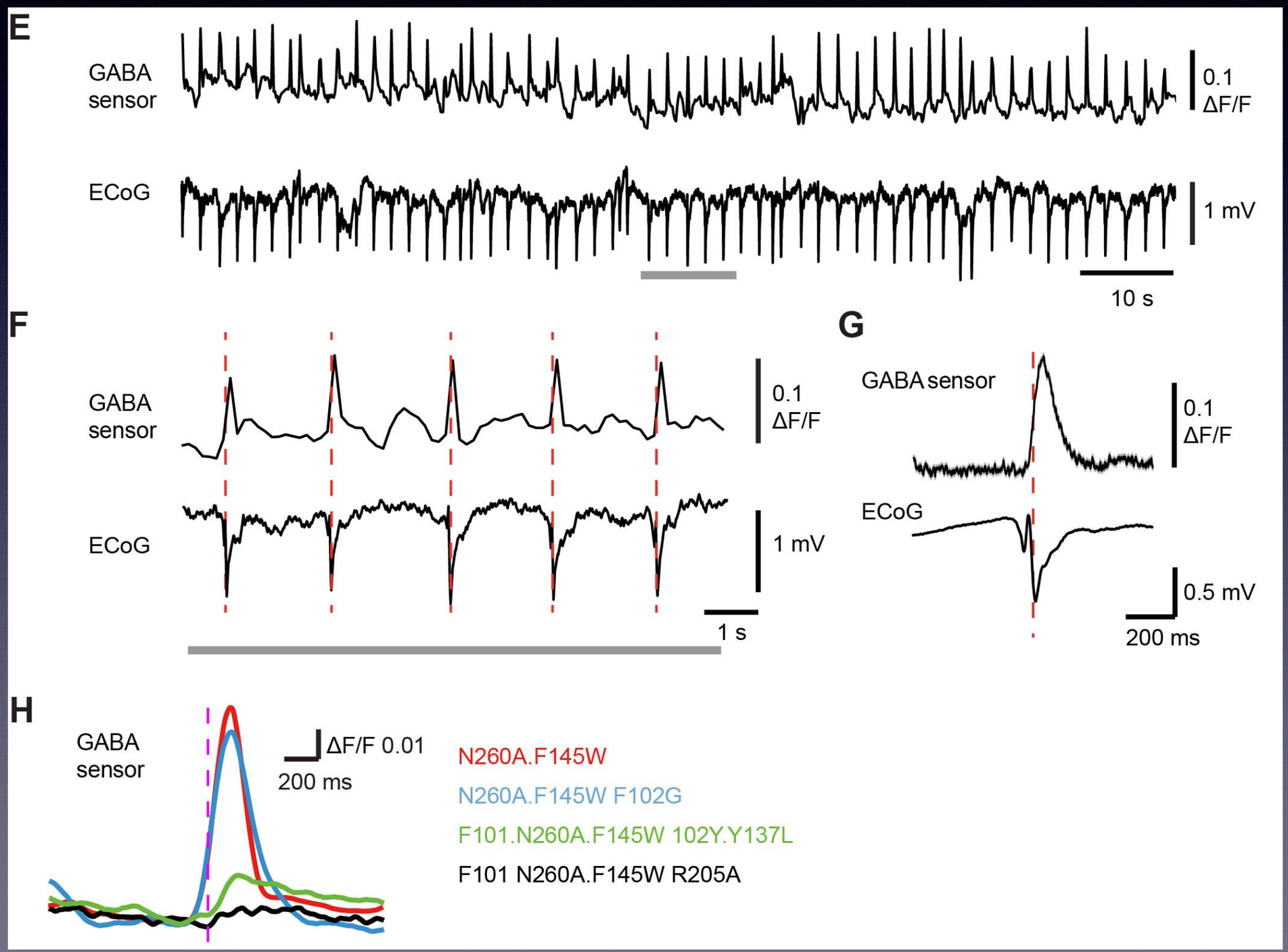


GABA release in mouse model of epilepsy



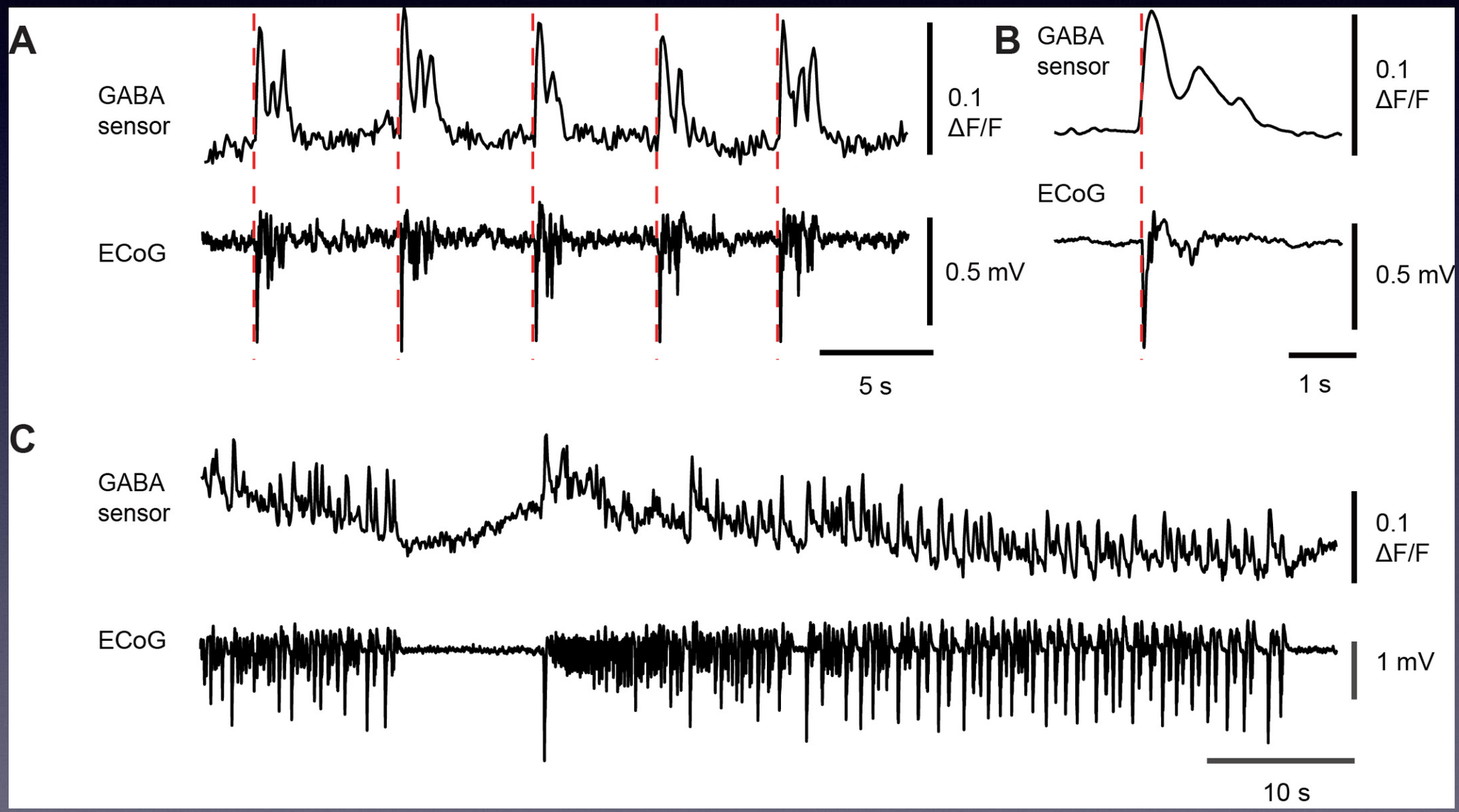
Dimitri Kullman's group @ UCL

GABA release in inter-ictal (non-seizure) spiking



Dimitri Kullman's group @ UCL

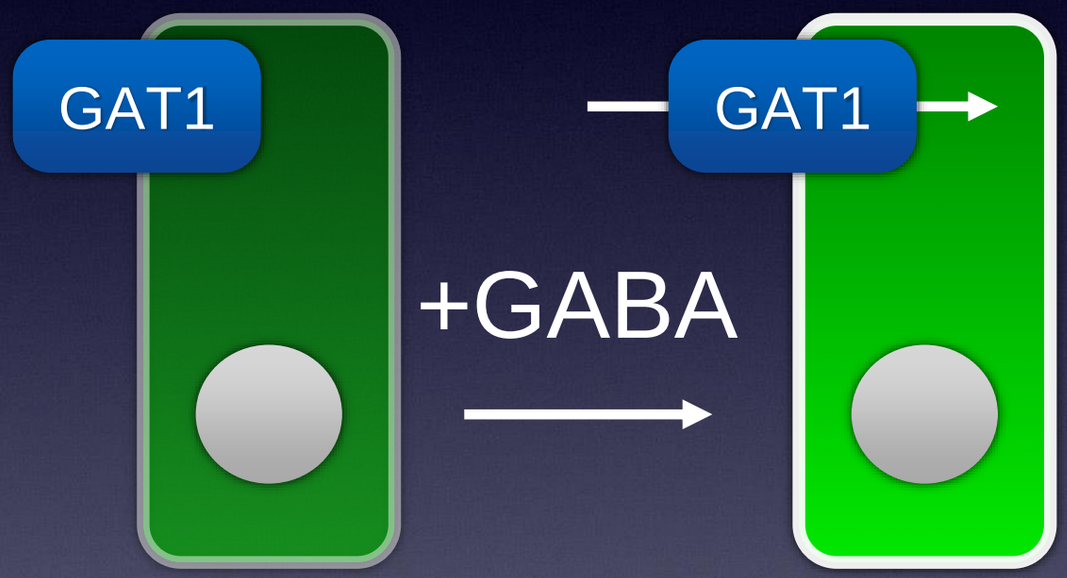
GABA release during seizures



iGABASnFR to detect cytosolic GABA transport

+GABA

extracellular signaling

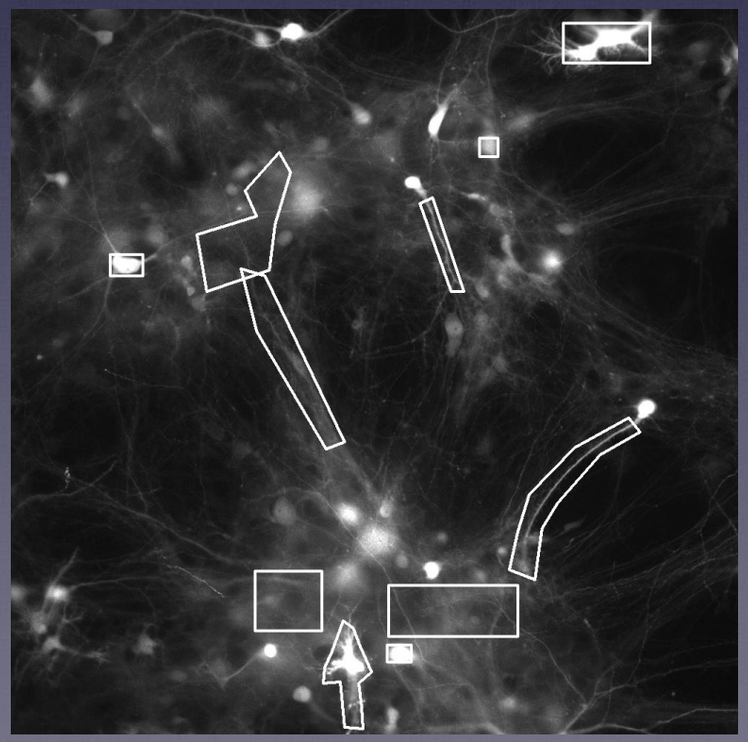


+GABA

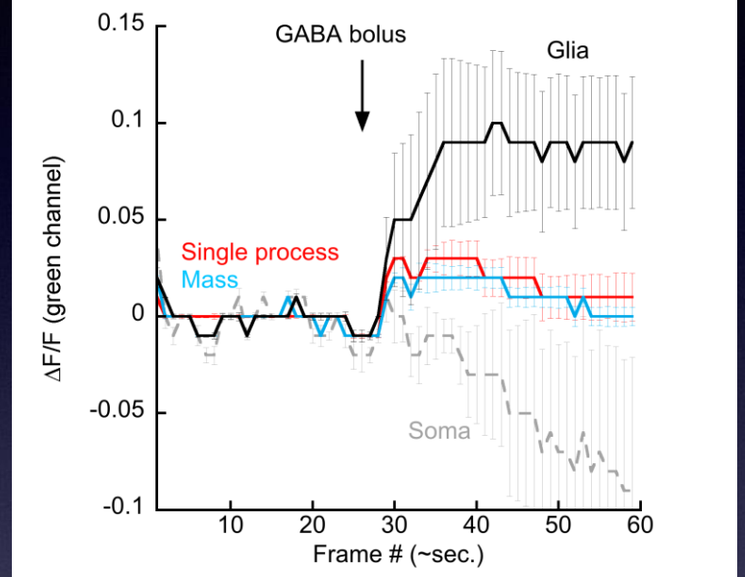
intracellular transport/metabolism

iGABASnFR to detect cytosolic GABA transport

Rat cortical culture
14 days post electroporation with cytosolic iGABASnFR.mRuby
imaged green (iGABASnFR)
imaged red (control FP)
Added bolus of GABA or Buffer



green



red